HILGARDIA

A Journal of Agricultural Science Published by the California Agricultural Experiment Station

Vol. 20

SEPTEMBER, 1950

No. 9

STEM ROT CAUSED BY PYTHIUM SPP. AND RHIZOCTONIA SOLANI^{1,2}

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INTRODUCTION

A DESTRUCTIVE root and stem disease of poinsettia (Euphorbia pulcherrima Willd.) has been prevalent in greenhouses at Encinitas, San Diego County, California, and in the San Francisco Bay region during the past fifteen years. In some seasons losses have been very heavy at Encinitas, where the bulk of the poinsettia cuttings used for potted plants by nurserymen for the Christmas trade are grown. Similarly, in nurseries in the San Francisco Bay region, where thousands of rooted cuttings from Encinitas are potted annually, growers have frequently lost 25 to 40 per cent of their crop. Because of the economic importance of this disease, an investigation has been conducted during the past ten years, the results of which are recorded here.

REVIEW OF LITERATURE

Peltier (1916)⁵ reported damping-off of poinsettia cuttings in a propagating box at Urbana, Illinois, in October, 1912. The causal fungus proved to be *Rhizoctonia solani* Kühn. Collar lesions which formed around the stem at the soil surface were 2 to 3 millimeters wide, somewhat depressed, and of a dark color.

Scott, Boillot, and Sullivan (1932) referred to damping-off of poinsettia cuttings in greenhouses where the sand contained organic matter and where ventilation was poor. No causal fungus was given.

Pythium ultimum Trow was stated by Middleton (1938) to be pathogenic to poinsettia cuttings. Later, in his monograph on the genus Pythium (1943), he listed the following species from this host: P. debaryanum Hesse, P. megalacanthum de Bary, P. oligandrum Drechs., P. perniciosum Serbinow, P. polymastum Drechs., and P. ultimum.

¹ Received for publication January 5, 1950.

² Contribution from the Division of Plant Pathology, Berkeley and Riverside. (Paper No. 641, University of California Citrus Experiment Station, Riverside, California.)

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In his contribution toward a host index of plant diseases in Oklahoma, Brown (1940) listed poinsettia as susceptible to infection by *Pythium* sp. Smith (1940) reported root rot and wilt of poinsettia plants caused by *Pythium debaryanum* and *P. ultimum*. The leaves of infected plants turned yellow and wilted and the fibrous roots became brown; these symptoms were

followed by the death of the plant. He suggested rooting poinsettias in sterilized sand or in fresh soil never before used for propagating plants of any kind, and cautioned against overwatering.

In New York, excellent control of rhizoctonia rot of poinsettia cuttings was obtained by dusting the surface of the sand with Fermate (Dimock, 1945).

Preston (1945) listed *Pythium debaryanum* as the cause of a stem rot of poinsettia in Oklahoma. Forsberg (1946), in Colorado, listed stem rot of poinsettia caused by *Rhizoctonia solani*. He recommended the use of sterilized sand in the cutting bench and the use of sterilized soil in planting the rooted cuttings.

SYMPTOMS OF THE DISEASE

Poinsettia cuttings placed in moist sand in greenhouse propagating benches at Encinitas may become infected before roots have formed. The stem of the cutting assumes a dark-brown, water-soaked appearance, extending above and below the soil line for a distance of one or more centimeters. Lesions may completely encircle the stem and cause it to become weak and constricted (plates 1 and 2). The foliage may become chlorotic if progress of the disease is slow. The lower, yellowed leaves abscise first and are followed by those higher on the stem. Yellowed leaves may remain flat before or after abscission, or the edges may curve upward along the midrib. If the disease progresses rapidly, the foliage wilts suddenly and the stem lesions cause the plant to lodge on the ground. New roots may form on cuttings before infection occurs on the stem. It is common to find fibrous roots and stems simultaneously showing discoloration, accompanied by yellowing, or sudden wilting without yellowing, of the foliage. The appearance of diseased plants is in marked contrast to that of healthy plants (plate 1, F). As the roots and stems become severely infected, the plants suddenly die.

The rooted cuttings produced in Encinitas are shipped to growers in the San Francisco Bay region. They are then transplanted to 2-inch pots, watered, and subjected to heavy shade and high humidity to offset the shock of pulling them from the sand in the propagation benches, shipping, and transplanting. After three days, the potted transplants are placed on benches in the greenhouse and receive normal watering. It is during the first few weeks after transplanting that most of the loss occurs. Root and stem infection, accompanied by sudden wilting of the foliage, may be so severe that hundreds of plants die daily.

In some instances the disease may occur after rooted cuttings have been shifted from small to large pots. If overwatered, or if unfavorable climatic conditions prevail, root and stem infection may occur and result in serious stunting or death of the plant. Occasionally, rooted cuttings, after the final transfer to large pots, grow very slowly between September and December. The foliage does not wilt or turn yellow; yet the root system, upon examina-

tion, is always found to be discolored and to be reduced in size in comparison with that of healthy plants. The infected plants are definitely stunted and seldom exceed 6 inches in height.

THE CAUSAL FUNGI

Isolation. Over a period of ten years isolations made on water or maltextract agar from the roots and stems of diseased unrooted and rooted cuttings of poinsettia from Encinitas, and from rooted cuttings and potted plants collected in various nurseries in the San Francisco Bay region, have consistently yielded *Pythium* spp. In 1947 and 1948, isolations yielded, also, cultures of *Rhizoctonia solani* Kühn (*Pellicularia filamentosa* [Pat.] Rogers). Of the *Pythium* species isolated, *P. ultimum* appeared with much greater frequency than did *P. debaryanum*, *P. irregulare* Buis., *P. oligandrum*, and *P. perniciosum*. Pure cultures were obtained from hyphal tips of the fungi in petri dishes.

Morphology. The sporangia of *P. debaryanum*, *P. irregulare*, and *P. ultimum* are similar in that they are typically subspherical, ovoid, or obovoid, and are most frequently terminal, though sometimes intercalary, and then usually ellipsoidal or prolate ellipsoidal in shape. Sporangia of *P. oligandrum* consist of contiguous spherical elements. Those of *P. perniciosum* are filamentous and undifferentiated from the mycelium.

The oögonia of $Pythium\ debaryanum\ and\ P.\ ultimum\ are\ somewhat\ alike,$ are similar to their sporangia, and possess a thin, smooth oögonial wall. Oögonia of $P.\ irregulare\ and\ P.\ oligandrum\ are\ terminal\ or\ intercalary\ and$ are more or less spherical in contour, with a thin wall beset with spines. The spines of $P.\ irregulare\ are\ few\ in\ number,$ of variable length and shape but usually of broad base and acuminate apex, and are straight or contorted. Spines of $P.\ oligandrum\ are\ numerous\ and\ of\ fairly\ regular\ length\ and\ shape,$ being conical and acutely tipped and measuring approximately 4μ long and 2μ wide at the base. The oögonia of $P.\ perniciosum\ are\ spherical,\ smooth\ and\ thin\ walled,\ and\ are\ usually\ acrogenous\ on\ short\ mycelial\ branches.$

The oöspores of all the Pythium species mentioned above are aplerotic.

Isolates of *Rhizoctonia solani* grown on potato dextrose agar produced a slight stroma-like layer on the agar surface, which became brown with age. Sclerotia were produced throughout the surface growth and were either distinct entities or amalgamated into groups which measured about 8 mm in diameter. The cultures appear to be similar to culture type C of Houston (1945).

Growth-Temperature Relations. The cardinal temperatures for growth of the several species of fungi isolated were determined to be as follows:

| | Temperature, °C | | |
|---------------------|-----------------|---------|---------|
| Species | Minimum | Optimum | Maximum |
| Pythium debaryanum | 1 | 28 | 37 |
| Pythium irregulare | | 28 | 37 |
| Pythium oligandrum | _ | 31 | 40 |
| Pythium perniciosum | | 31 | 37 |
| Pythium ultimum | | 28 | 37 |
| Rhizoctonia solani | | 25 | 31-34 |

Values for *Pythium* spp. conform with those given by Middleton (1943) for isolates of the species obtained from other hosts in various localities. Values for *Rhizoctonia solani* agree with those given by Houston (1945) for

culture type C.

Pathogenicity. Pure cultures of Pythium debaryanum, P. irregulare, P. oligandrum, P. perniciosum, P. ultimum, and Rhizoctonia solani were used in the infection experiments. Inoculum was prepared by growing the Pythium isolates on a mixture of sterilized, moistened cracked wheat and oats, and by growing isolates of Rhizoctonia solani on sterilized, moistened cracked wheat.

Inoculum was added in uniform quantity to autoclaved soil in 5- or 6-inch pots, each containing a single, young, healthy poinsettia plant, of the varieties Oak Leaf, Mrs. Paul Ecke, Henrietta Ecke, or Albert Ecke, in a manner designed to avoid wounding the roots or stems. Sterilized cracked wheat and oats, or cracked wheat alone, were used for the noninoculated controls. The plants were grown in a greenhouse at temperatures ranging from 70° to 80° F during the day and about 65° at night. They were watered heavily each day to provide favorable conditions for infection.

Three lots of 32 healthy poinsettia plants were inoculated with *Pythium ultimum*, the species most commonly isolated from naturally infected plants grown at Encinitas and in the San Francisco Bay region. Each lot was inoculated with one of three isolates of this species. Of the 96 plants inoculated, 65 became infected in incubation periods ranging from 8 to 19 days. All isolates produced similar symptoms in similar incubation periods (plate 3, A, B). Infected plants usually died within a month after inoculation.

Two lots of 42 healthy poinsettia plants were inoculated with isolates of *Pythium perniciosum* and *P. debaryanum*, respectively. *P. perniciosum* induced infection in 17 plants in an incubation period of 15 to 20 days. *P. debaryanum* caused 25 plants to become infected in a period of 14 to 23 days. As with *P. ultimum*, infected plants usually died within a month after inoculation.

Two species of *Pythium* infrequently isolated from infected poinsettias were also tested. One lot of 24 healthy plants was inoculated with *P. irregulare*, and a second lot with *P. oligandrum*. After 14 days, one inoculated poinsettia plant became infected by *P. irregulare*; no infection was obtained with *P. oligandrum*. The two series of inoculated plants were held for one month before discarding.

Two isolates of *Rhizoctonia solani* were tested on two lots of 24 healthy poinsettia plants each. One isolate infected 23 plants and the other 20 plants, the incubation period ranging from 6 to 8 days (plate 4, A, B, C). Infected plants died within 10 days after inoculation.

Approximately 124 healthy poinsettia plants were maintained as controls while the inoculation tests were in progress and all remained healthy (plate 3, C).

Reisolations were made from all infected plants, and the reisolates proved pathogenic when tested on healthy plants. Symptoms on artificially infected plants were identical with those of naturally infected plants.

CONTROL OF THE DISEASE

It has been common practice at Encinitas to use the same sand year after year in the propagating benches in which poinsettia cuttings are placed. At first there was little or no loss, but as the pathogenic fungus flora increased, losses became progressively greater. Sterilization equipment was not available; hence other means had to be used to combat the disease. Accordingly in the spring of 1947, all sand was removed from the propagating benches, which were then thoroughly cleaned and filled with new, apparently clean sand of proper texture. The surface of the sand was thoroughly dusted with Ferbam, which later was raked and watered into the sand. Cuttings. also, were dusted with Ferbam powder as an additional means of protection. In the late summer of 1947 an examination of the thousands of cuttings being prepared for shipment, and of those still in the propagating benches, revealed only two infected plants. Likewise, in 1948, only a trace of the disease was found at Encinitas. Thus, the rooted poinsettia cuttings were healthy, with bright roots and clean stems, when shipped from Encinitas to various nurseries in the San Francisco Bay region. This fact was confirmed by inspection of many shipments of rooted cuttings, amounting in the aggregate to thousands of plants, after they arrived at the nurseries for transplanting during the summer and fall of 1947 and 1948.

It is likely that before control of the disease was obtained in the propagating benches at Encinitas, both infected and healthy rooted cuttings were shipped to the San Francisco Bay region. Losses were heavy after transplanting, regardless of the care and attention given to the transplants. But the incidence of disease was not entirely due to infected cuttings. Very few nurseries sterilize their potting soil, either with steam or with chemicals; hence much of the infection can be ascribed to local conditions. This proved self-evident, commencing with the season of 1947, when only healthy rooted cuttings arrived in the Bay region: heavy losses still occurred after transplanting, and observations over a two-year period indicate conclusively that the healthy rooted cuttings became infected after transplanting into small pots filled with nonsterilized soil. Steam or chemical sterilization of the potting soil and pots appears to be the most promising means of controlling this disease in the greenhouses.

In one nursery in the San Francisco Bay region cuttings are rooted by placing them individually in 2-inch pots of steam-sterilized sand. When rooted, the cuttings are removed from the pots, the sand is shaken from the root system, and the plants are immediately placed in larger, permanent pots filled with clean, but not necessarily steam-sterilized, soil. Very little loss has occurred at any stage of growth, from the cutting period until the plants mature, just before Christmas.

To summarize, observations in commercial greenhouses indicate that the disease is favored in its development and spread by one or more of several factors, namely: unclean sand in the propagating benches, nonsterilized or

[&]quot;Ferbam" is the common name for ferric dimethyl dithiocarbamate selected by the Subcommittee on Fungicide Nomenclature of the American Phytopathological Society, in cooperation with the Interdepartmental Committee on Pest Control, and filed with the Trade-Mark Division of the United States Patent Office.

ordinary potting soil, excessive moisture, excessive humidity after transplanting, crowding of the plants after potting, inadequate ventilation, and chilling of the plants by air temperatures below 60° F.

DISCUSSION

Commencing May 1, more than one-half million poinsettia cuttings are rooted annually at Encinitas, in preparation for the Christmas trade. These cuttings are shipped to pot-plant nurseries in California and throughout the nation. During the summer months, while shipment is in progress, at least 150,000 cuttings are kept constantly on hand in the propagating benches. These facts emphasize the importance of this industry in southern California and what might conceivably happen if a disease such as the one discussed in this paper should become epidemic. From 1938 through 1946, heavy losses from this disease were sustained at Encinitas. Subsequently, effective methods of control were devised.

Losses have been correspondingly heavy at the pot-plant nurseries from 1938 to date, with most of the damage occurring immediately after the rooted cuttings are potted in soil. Most nurseries sustain heavy losses annually throughout this critical period, and it apparently makes little difference whether cuttings arrive early in the season (July), in midseason (August), or late (September). A few growers report losses only in late shipments. Most plants become infected during the 3 to 7 days after transplanting, while they are being held in the benches, covered with stiff paper, or in cloth cages. Others become infected after they are placed on open benches in the greenhouse. Poinsettia transplants are covered to reduce transpiration while the root system is becoming established in the soil, although it is recognized that this practice provides excessive humidity and increased air temperature, both of which are favorable to infection.

Poinsettia growers generally agree that the temperatures most favorable for poinsettia culture range from 74° to 80° F during the day and from 60° to 64° at night. Sometimes growers fail to regulate the air temperatures properly in the greenhouses, and at night the temperature may drop below 60°, thus chilling the plants.

Overwatering of the plants has been one of the primary contributing factors associated with high incidence of the disease. Within recent years potplant growers have learned that they have less infection if they use less water.

It is believed that losses sustained in pot-plant nurseries in the San Francisco Bay region since 1947 are attributable, not to infected cuttings shipped from Encinitas, but rather to the fact that ordinary soil containing pathogenic fungi has been used as the potting medium. As previously stated, rooted cuttings shipped from Encinitas since 1947 have been clean and free from disease. With proper equipment the potting soil could be treated at moderate expense with steam or chemicals, thus eliminating, in large part, the annual losses which afflict the growers.

The poinsettia varieties Oak Leaf, Mrs. Paul Ecke, Henrietta Ecke, and Albert Ecke were tested by inoculation in the greenhouse and found highly susceptible to infection. This agrees with nursery observations. No infection has been found on the variety Indianapolis, grown on a limited scale in some

nurseries. Material was not available for testing this relatively new variety. In a casual or macroscopic examination of infected plants, it is impossible to determine which fungus is involved. Laboratory cultural tests are required. In cultures from more than 1,000 infected plants since 1938, only one species of *Pythium* has been found to occur in any single infected plant. *Rhizoctonia*

solani also occurs alone and not in combination with any species of Pythium. The three species of Pythium, P. debaryanum, P. perniciosum, and P. ultimum, and Rhizoctonia solani are the fungi responsible for plant loss in the course of plant establishment. The stunting of established plants is associated only with infection by P. ultimum.

ciated only with infection by P. ultimum

SUMMARY

A root and stem disease of poinsettia (*Euphorbia pulcherrima* Willd.) has been prevalent at Encinitas, San Diego County, and in the San Francisco Bay region of California for the past fifteen years.

Symptoms of the disease are: wilting of the plant (with or without chlorosis), abscission of lower leaves, dark-brown stem lesions which may completely girdle the stem and cause it to shrivel and lodge on the ground, and dark-brown roots. Infected plants usually die quickly. Occasionally these plants survive until the Christmas season, but they are stunted, seldom exceeding 6 inches in height, and the root system is dark brown.

The principal environmental factors favoring the disease are excessive soil moisture and humidity, air temperatures that are above 80° F or below 60° F,

and overcrowding of the plants.

The causal organisms have been identified as *Pythium debaryanum* Hesse, *P. perniciosum* Serbinow, *P. ultimum* Trow, and *Rhizoctonia solani* Kühn. *P. irregulare* Buis. is mildly pathogenic, and *P. oligandrum* Drechs. is non-pathogenic, to poinsettia cuttings.

Infection was obtained in greenhouse experiments by adding the four fungi separately to the wet, autoclaved soil of different lots of potted plants. The incubation periods ranged from 8 to 23 days for *Pythium* spp., and from

6 to 8 days for Rhizoctonia solani. All the infected plants died.

It is impossible to determine macroscopically which of the four causal fungi may be present in diseased plants. The fungi are found separately and not in combination, and this suggests that although the disease involves a complex of organisms there is no interaction of fungi.

The poinsettia varieties Oak Leaf, Mrs. Paul Ecke, Henrietta Ecke, and Albert Ecke were tested and found to be highly susceptible to infection. This

supports observations made in nurseries.

The disease may be controlled in the propagation benches by steam or chemical sterilization of the sand in which the cuttings are to be rooted. Ferbam (ferric dimethyl dithiocarbamate) has given good results when applied as a dust to the surface of the sand and dusted on the cuttings immediately before placing them in the sand. The use of sterilized pots and either steam- or chemically sterilized potting soil at transplanting time is a prerequisite for maintaining the health of the plants. Proper ventilation, air temperature, humidity, and watering are also important in avoiding disease.

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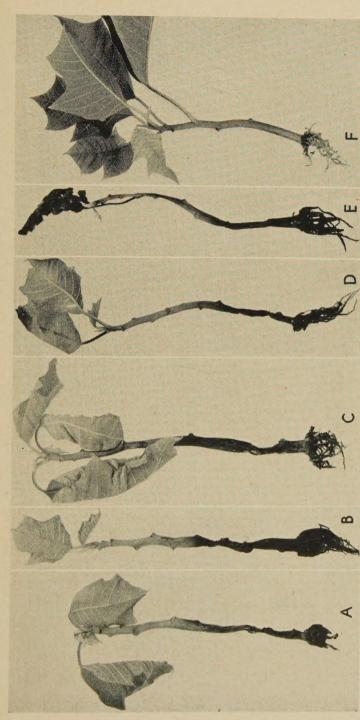
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dark brown color, and are unable to function. Dark-brown stem lesions extend several inches above the soil line. The leaves of infected cuttings Plate 1. Natural infection of rooted cuttings of poinsettia (Euphorbia pulcherrima) by Pythium ultimum: A-D, infection has destroyed most of the fibrous root system of these cuttings; the few remaining rootlets are completely invaded by the fungus, as indicated by their wilt, often becoming chlorotic as the stem cankers advance upward, and abscise. Stem tissues become distorted and constricted, cuttings collapse and die. E, a rooted cutting whose tissues are almost completely invaded by the fungus. F, a healthy rooted cutting.



Plate 2. Natural infection of rooted cuttings of poinsettia (Euphorbia pulcherrima) by Rhizoctonia solani: A-C, the root systems of these cuttings have been completely destroyed, and stem cankers have progressed upward above the soil line to involve most of the stem tissues. Leaves of infected cuttings wilt, sometimes becoming chlorotic, and abscise from the stems as infection proceeds.

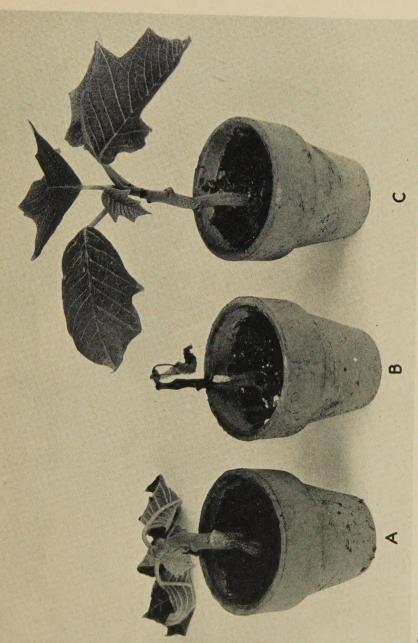


Plate 3. Artificial infection of rooted cuttings of poinsettia (Euphorbia pulcherrima) grown in pots and inoculated in the greenhouse by adding wheat-oats cultures of Pythium ultimum to the soil: A, early stage of infection, showing wilting and lodging of the plant after 11 days; B, complete infection and death of the plant, without lodging, after 18 days; C, healthy control plant which was treated with a mixture of sterile oats and wheat.

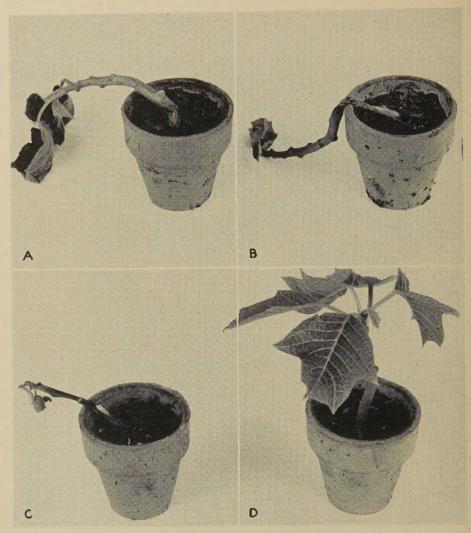


Plate 4. Artificial infection of rooted cuttings of poinsettia ($Euphorbia\ pulcherrima$) grown in pots and inoculated in the greenhouse by adding wheat cultures of $Rhizoctonia\ solani$ to the soil: A-C, collapsed young potted rooted cuttings six days after inoculation; D, healthy control plant which was treated with a mixture of sterile wheat.